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Tumour Kinetics, Response to Chemotherapy and Survival in Primary Ovarian Cancer

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The analysis of thymidine labelling index (TLI) in relation to clinico-pathological variables and survival was carried out in 111 ovarian cancer patients. The significance of TLI in predicting response to aggressive first line chemotherapy regimens was examined. The overall median TLI value of 1.8% was used as a cut-off to discriminate slowly from highly proliferating cancers. 94 patients entered into two consecutive randomised trials, and were treated with six courses of cisplatin-based chemotherapy with or without doxorubicin. A significantly higher objective response of 60% was reported in the subset of patients with TLI > 1.8% as compared to 35% in patients with TLI ≤ 1.8% ($P = 0.03$). In addition, patients achieving complete response had tumours with median TLI of 3.8% as compared to 2.4% for partial responders, 1.5% for patients with stable disease and 1.7% for those with progressive disease. A significant increase in tumour kinetics was observed in advanced cancers ($P = 0.001$), more undifferentiated tumours ($P = 0.02$) and postsurgical residual disease greater than 2 cm ($P = 0.04$). In univariate analysis, TLI failed to influence significantly clinical outcome: 26 versus 32 months median survival time for patients with high and low tumour TLI, respectively. In the Cox's regression model, the only independent prognostic variables were performance status and amount of residual disease after primary surgery ($P = 0.000$).

Key words: tumour kinetics, chemotherapy, survival, ovarian cancer
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INTRODUCTION

THE NATURAL history and management of ovarian cancer have been extensively covered in numerous articles and reviews during the last few decades [1–3]. Main emphasis has been given to the comparison of different regimens of chemotherapy to select the optimal treatment, in terms of response rates and survival [4–7]. Hence, the identification of independent prognostic factors able to predict the outcome of ovarian cancer has been widely pursued. The prognostic variables might, in fact, provide a framework for appropriate selection of therapy according to subsets of patients at different risk of death [8–10].

At present, the amount of residual disease after primary surgery and performance status are the most consistently independent predictors of survival [8–11]. However, biological parameters have not been thoroughly investigated, and their role in defining prognosis remains unclear. Experimental and clinical investigations have provided evidence that higher tumour cell kinetics are strictly related to chemosensitivity [12–15]. This holds true in primary untreated tumours and before drug resistance develops [16]. In a preliminary investigation, we demonstrated that the probability of achieving an objective response to aggressive combination chemotherapy was significantly related to high proliferative activity of the primary tumour [17]. At that time, we suggested a possible role of tumour kinetics in selecting poor risk patients potentially benefiting from more aggressive treatment strategies. In this paper, we report the updated results of a larger series of patients enrolled on to consecutive clinical trials of the Italian North-West Oncologic Cooperative Group, GONO, from 1982 to 1991 [17,18]. The biological and clinical significance of thymidine labelling index (TLI) is analysed and discussed.

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PATIENTS AND METHODS

Patients and treatment

The TLI of the primary tumour was evaluated at the time of first surgery in 111 previously untreated ovarian cancer patients. Patient characteristics are summarised in Table 1. FIGO classification was adopted to define extent of disease and grade of tumour differentiation. Clinical response to treatment was assessed according to WHO criteria. Second look laparatomy was performed in patients with no clinical evidence of disease after six courses of cisplatin-based chemotherapy to define the pathological response. Criteria for surgical staging and second look laparatomy procedures have been described previously [18]. The majority of patients (84.7%) entered into two consecutive randomised trials and were treated according to the following regimens: PAC or CAC = cisplatin 50 mg/m^2 or carboplatin 200 mg/m^2 + cyclophosphamide 600 mg/m^2 + doxorubicin 45 mg/m^2 intravenous (i.v.) day 1 every 28 days for six courses (62 patients); PC = cisplatin 50 mg/m^2 + cyclophosphamide 600

Table 1. TLI and clinicopathological characteristics of patients

Covariate	Patients no.	TLI median (range)	Kruskal–Wallis statistics	P value
Overall	111	1.8 (0.1–28.0)		
Age (years)				
≤51	33	1.5 (0.2–24.0)		
52–61	39	2.3 (0.2–17.8)	3.91	NS
>61	39	1.9 (0.1–28.0)		
ECOG PS				
0	54	1.5 (0.2–24.0)	1.13	NS
≥1	57	2.0 (0.1–28.0)		
Histology				
Endometrial	13	0.7 (0.2–10.7)		
Mucinous	12	1.5 (0.2–11.8)		
Undifferentiated	12	2.0 (0.2–9.20)	8.11	NS
Serous	60	2.4 (0.1–24.0)		
Others	13	2.1 (1.0–28.0)		
Residual disease				
≤2 cm	52	1.5 (0.2–17.8)	3.92	0.04
>2 cm	59	2.4 (0.1–28.0)		
Grading*				
1	15	1.0 (0.4–2.50)		
2	40	1.6 (0.2–24.0)	7.56	0.02
3	50	2.5 (0.1–17.8)		
Stage				
I+II	34	1.2 (0.2–17.8)		
III	58	1.8 (0.1–24.0)	13.3	0.001
IV	19	2.9 (0.4–28.0)		

PS, performance status; NS, non-significant. * Grading was not determined in 6 patients.

mg/m^2 i.v. day 1 every 28 days for six courses (32 patients). 10 patients (9%) received melphalan 6 mg/m^2 every day for 5 consecutive days every 6 weeks until disease progression.

7 patients received no treatment either because they refused or for medical reasons.

TLI

Cell kinetics, as the percentage of thymidine labelled cells in DNA synthesis over all the tumour population, were evaluated on tumour specimens at the time of first surgery and carried out according to the procedure described previously [19].

Statistical analyses

The association between clinico-pathological characteristics of patients and TLI was investigated by Kruskal–Wallis test statistics, for two or more than two groups, and tested for significance according to the χ^2 test ($P = 0.05$). The effect of each covariate on patients survival was investigated by using the Kaplan–Meier step function [20].

Differences between survival curves were tested according to the log rank test. Cox's multiple regression analysis [21] was used to study the effect of each covariate on patient survival while adjusting for the other covariates, and to identify prognostic variables through a backward stepwise procedure. Covariates significantly related to survival were identified according to the likelihood ratio test as allowed by the BMDP statistical software [22].

RESULTS

The overall median TLI value was 1.8% (range 0.1–28.0). The relationship between TLI and the main clinico-pathological characteristics is reported in Table 1. TLI values were not related to age, ECOG performance status (PS) or histology. A significant increase in tumour proliferative activity was observed in residual disease after primary surgery greater than 2 cm ($P = 0.04$), more undifferentiated tumours ($P = 0.02$), and advanced stages ($P = 0.001$).

Univariate survival analyses of prognostic factors are reported in Table 2. PS, residual disease, histological diagnosis, age, grading and stage of disease were statistically significant predictors of survival. On the contrary, TLI failed to significantly influence patients outcome with 26 months median survival time for patients with a tumour TLI above 1.8% compared with 32 months for those with TLI values equal to or below 1.8% (Figure 1).

74 of the 94 patients who entered two randomised trials were evaluable for response. Table 3 reports the relationship between TLI value and treatment response in those patients surgically re-explored (33 patients) or with clinically measurable disease (41 patients). An objective response (complete + partial response) was observed in 35% of patients with low TLI compared to 60% of patients with high TLI ($P = 0.03$). Moreover, median TLI, according to treatment response, was 3.8% (range 0.6–13.5) for complete response, 2.4% (range 0.2–24.0) for partial response, 1.5% (range 0.1–11.8) for stable disease and 1.7% (range 0.2–28.0) for progressive disease.

Table 4 summarises the results obtained according to the Cox's multiple regression analysis on the 94 patients. The estimated relative risk (RR), 95% confidence interval (CI) and the computed probability for each covariate included in the final model are reported.

Performance status and postsurgical residual disease were identified as the most important predictors of survival ($P = 0.000$).

Table 2. Univariate survival analysis

Covariate	Patients no.	Observed/ expected	*P*	Median survival (months)
ECOG PS				
0	54	1	0.0000	68
≥1	57	3.1		14
Residual disease				
≤2 cm	52	1	0.0001	*
>2 cm	59	2.6		15
Histology				
Endometrial	13	1		*
Mucinous	12	1.4		27
Serous	60	1.7	0.009	32
Others	14	2.2		9
Undifferentiated	12	4.9		9
Age (years)				
≤51	33	1.2		28
52–60	39	1	0.015	41
≥61	39	2.1		14
Grading				
1	15	1		*
2	40	1.4	0.024	33
3	50	1.9		19
Stage				
I+II	34	1		*
III	58	2.3	0.03	27
IV	19	4.6		12
TLI				
≤1.8	57	1	0.45	32
>1.8	54	1.1		26
Therapy				
PC	32	1		34
PAC/CAC	62	1.4	0.60	30
Melphalan	10	1.5		4

PS, performance status; TLI, tumour labelling index. * Median survival time not reached.

Patients with performance status above 1 and postsurgical residual disease greater than 2 cm, had 1.66 and 1.46 relative risks of death, respectively, compared to patients with ECOG PS equal to 0 and residual disease less than 2 cm.

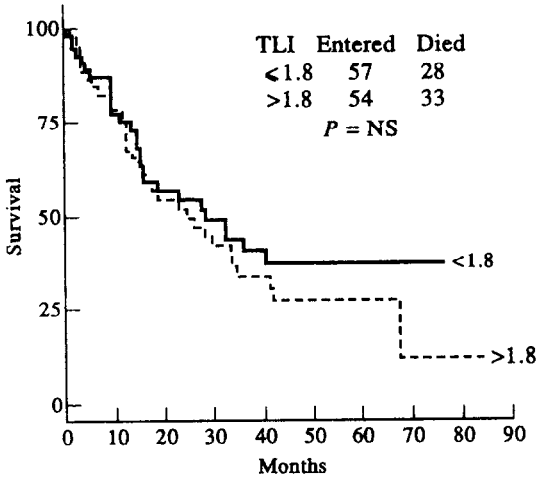

Fig. 1. Relationship between TLI and survival in patients with primary ovarian cancer.

Table 3. TLI and response rate

Response	TLI Median (range)	TLI≤1.8 No.	(%)	TLI>1.8 No.	(%)
CR	3.8 (0.6–13.5)	5	35	12	60
PR	2.4 (0.2–24.0)	7		12	
SD	1.5 (0.1–11.8)	10	65	5	40
PD	1.7 (0.2–28.0)	12		11	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; TLI, tumour labelling index. CR+PR versus SD+PD, $P = 0.03$.

Table 4. Multivariate analysis by Cox's model

Covariate	RR	95% CI	*P* (test for trend)
ECOG PS			
0	1		
≥1	1.66	1.01–2.09	0.000
Residual disease			
≤2 cm	1		
>2 cm	1.46	1.02–2.72	0.000

RR, relative risk; CI, confidence interval; PS, performance status.

DISCUSSION

In the present report, the relationship between tumour TLI and several clinicopathological factors in patients with primary ovarian cancer has been investigated. Moreover, the impact of TLI in predicting survival and response to combination chemotherapy has been further assessed. Advanced cancers, undifferentiated tumours and post-surgical residual disease greater than 2 cm were significantly associated with a higher tumour growth rate. These parameters are known prognostic factors of tumour aggressiveness, as also confirmed by the present univariate analyses. Thus, cell kinetics appear indicative of the degree of malignancy.

The findings of this study indicate that TLI failed to significantly predict survival both in univariate and multivariate analyses. Nevertheless, analysis of tumour kinetics revealed a direct association with clinical or surgical response to chemotherapy. Our results, in fact, showed a 60% objective response in the group of patients with higher proliferating tumours compared to 35% in the slower proliferating group. This evidence was more striking when we looked at the distribution of median TLIs according to response rate, with a median value of 3.8% in the subset of patients who achieved complete response. Therefore, our data demonstrate that tumour cell kinetics may be relevant in predicting response to combination chemotherapy, despite the fact that statistical significance in survival analysis is lacking.

Only a relative number of studies, taking into account the relationship between cell kinetics and survival in ovarian cancer, are reported in the literature, and some support our findings. Erba and colleagues investigated the role of flow cytometry, clinico-pathological prognostic variables and patients outcome, and found that the percentage of S-phase cells did significantly correlate with higher DNA aneuploidy and poorly differentiated tumours, although the association with survival was statistically non-significant [23].

More recently, Silvestrini and colleagues [24], who studied 85 advanced ovarian cancers, did not find a significant relationship between tumour proliferative activity and survival in patients treated with polychemotherapy regimens; the prognostic role of TLI was maintained in a small subset of patients submitted to monochemotherapy, further suggesting that aggressive chemotherapy regimens are better at controlling higher proliferating neoplasms. In addition, it is worth emphasising that, in untreated tumours, the prognostic significance of TLI is likely to be affected by aggressive chemotherapy, but in chemoresistant patients, the kinetic prognostic importance is apparent. In fact, our recent study on refractory or relapsing ovarian cancer demonstrated that, when drug resistance occurred, patients with low TLI tumours survived longer than did patients with high TLI [25].

In our opinion, this investigation indicates that (a) chemotherapy response rate is significantly associated to the growth aggressiveness of the primary untreated tumours; (b) in this selected series of patients, known clinico-pathological variables confirm their importance in the univariate and multivariate analyses.

In conclusion, TLI may predict probability of response to aggressive cisplatin-based chemotherapy in untreated ovarian cancer. In addition, tumour kinetics may be helpful in defining groups of patients at poor risk who might benefit from further therapeutic modalities.

1. Ozols RF, Hamilton TC, Hoskins WJ, Bast RC, Young RC. Summary of symposium: biology and therapy of ovarian cancer. *Semin Oncol* 1991, **18**, 297–306.
2. Perez RP, Godwin AK, Hamilton TC, Ozols RF. Ovarian cancer biology. *Semin Oncol* 1991, **18**, 186–204.
3. Richardson G, Scully R, Nikruy N, Nelson JH. Common epithelial cancer of the ovary. *N Engl J Med* 1985, **312**, 415–24, 474–483.
4. Cyclophosphamide plus cisplatin versus cyclophosphamide, doxorubicin and cisplatin chemotherapy of ovarian carcinoma: a meta-analysis by the Ovarian Cancer Meta-Analysis Project. *J Clin Oncol* 1991, **9**, 1668–1674.
5. MRC Ginecological Working Party. An overview in the treatment of advanced ovarian cancer. *Br J Cancer* 1990, **61**, 495–496.
6. Conte PF, Bruzzone M, Chiara, *et al.* A randomized trial containing platinum plus cyclophosphamide versus platinum, doxorubicin and cyclophosphamide in advanced ovarian cancer. *J Clin Oncol* 1986, **4**, 965–971.
7. Gruppo Interregionale Cooperativo Ginecologico Oncologico. Randomized comparison of cisplatin with cyclophosphamide, cisplatin and with cyclophosphamide, doxorubicin, cisplatin in advanced ovarian cancer. *Lancet* 1987, **2**, 353–359.
8. Van Houwelingen JC, Ten Bokkel Huinink WW, Van der Burg MEL, Van Oosterom AT, Neijt JP. Predictability of the survival of patients with advanced ovarian cancer. *J Clin Oncol* 1989, **7**, 769–773.
9. Omura GA, Brady MF, Homesley HD, *et al.* Long-term follow up and prognostic factor analysis in advanced ovarian carcinoma: the gynecologic oncology group experience. *J Clin Oncol* 1990, **9**, 1138–1150.
10. Marsoni S, Torri V, Valsecchi MG, *et al.* Prognostic factors in advanced epithelial ovarian cancer. *Br J Cancer* 1990, **62**, 444–450.
11. Dembo AJ, Davy M, Stewing AE, Berle EJ, Bush RS, Kjorstad K. Prognostic factors in patients with stage I epithelial ovarian cancer. *Obstet Gynecol* 1990, **75**, 263–273.
12. Drewinko B, Patchen M, Yang LY, Barlogie B. Differential killing efficacy of twenty antitumor drugs on proliferating and nonproliferating human tumor cells. *Cancer Res* 1981, **41**, 2328–2333.
13. Conte PF, Alama A, Favoni R, Trave F, Rosso R. Nicolin A. Timed sequential chemotherapy following drug-induced kinetic recruitment in refractory ovarian cancer. *Eur J Cancer Clin Oncol* 1984, **20**, 1039–1043.
14. Conte PF, Alama A, Bertelli G, *et al.* Chemotherapy with estrogenic recruitment and surgery in locally advanced breast cancer: clinical and cytokinetic results. *Int J Cancer* 1987, **40**, 490–494.
15. Valeriote FA, Edelstein MB. The role of cell kinetics in cancer chemotherapy. *Semin Oncol* 1977, **4**, 217–226.
16. Fojo A, Hamilton TC, Young RC, Ozols RF. Multidrug resistance in ovarian cancer. *Cancer* 1987, **60**, 2075–2080.
17. Conte PF, Alama A, Rubagotti A, *et al.* Cell kinetics in ovarian cancer: relationship to clinico-pathologic features responsiveness to chemotherapy and survival. *Cancer* 1989, **64**, 1188–1191.
18. Conte PF, Bruzzone M, Carnino, *et al.* Carboplatin, doxorubicin and cyclophosphamide versus cisplatin, doxorubicin and cyclophosphamide: a randomized trial in stage III–IV epithelial ovarian carcinoma. *J Clin Oncol* 1991, **9**, 658–663.
19. Alama A, Costantini M, Repetto L, *et al.* Thymidine labelling index as prognostic factor in resected non-small cell lung cancer. *Eur J Cancer* 1990, **26**, 622–625.
20. Kaplan EL, Meier P. Non parametric estimation from incomplete observation. *J Am Stat Assoc* 1958, **53**, 457–481.
21. Cox DR. Regression models and life tables. *J R Stat Soc B* 1972, **34**, 187–191.
22. Dixon WJ, Brown MB, Engelman L. *BMDP Statistical Software.* Berkeley, University of California Press, 1988.
23. Erba E, Ubezio P, Pepe S, *et al.* Flow cytometric analysis of DNA content in human ovarian cancers. *Br J Cancer* 1989, **60**, 45–50.
24. Silvestrini R, Daidone MG, Valentinis B, *et al.* Potential of cell kinetics in the management of patients with ovarian cancers. *Eur J Cancer* 1992, **28A**, 386–390.
25. Alama A, Merlo F, Chiara S, *et al.* Prediction of survival by thymidine labelling index in patients with resistant ovarian carcinoma. *Eur J Cancer* 1992, **28A**, 1079–1080.